

PMRA Submission Number: 2008-0431
PMRA Document ID: 1547199

EPA MRID Number: 47127903

DATA EVALUATION RECORD
ACUTE LC₅₀ TEST WITH AN ESTUARINE/MARINE SHRIMP
§72-3(C)

Data Requirement:

PMRA Data Code	9.4.2
EPA DP Barcode	DP349851
OECD Data Point	IIA 8.11.1
EPA MRID	47127903
EPA Guideline	OPPTS 850.1035

1. **CHEMICAL:** Saflufenacil PC Code No.: 118203

2. **TEST MATERIAL:** BAS 800 H Purity: 93.8%

3. **CITATION**

Authors: Blankinship, A.S., T.Z. Kendall, H.O. Krueger and C. Holmes
Title: BAS 800 H: A 96-Hour Flow-Through Acute Toxicity Test with the Saltwater Mysid (*Americamysis bahia*)
Study Completion Date: October 26, 2007
Laboratory: Wildlife International, Ltd., Easton, MD
Sponsor: BASF Corporation, Research Triangle Park, NC
Laboratory Report ID: 147A-212C
MRID No.: 471279-03
DP Barcode: DP349851

4. **REVIEWED BY:** John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature:  **Date:** 03/24/08

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental, Inc.

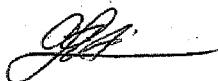
Signature:  **Date:** 04/04/08

5. **APPROVED BY:** Primary Reviewer: Anita Pease, Senior Biologist, U.S. EPA

Signature:  **Date:** 06/09/09

Secondary Reviewer: Ann Lee, HC-PMRA-EAD

Signature:



Date: 06/09/09

Secondary Reviewer: Farzad Jahromi, DEWHA-APVMA

Signature:



Date: 06/09/09

6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to shrimp. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. EXECUTIVE SUMMARY

In a 96-h flow-through study, juvenile saltwater mysid, *Americamysis bahia*, were exposed to nominal saflufenacil (BAS 800 H) concentrations of 0 (negative and solvent control), 1.3, 2.2, 3.5, 6.0, and 10 mg a.i./L. TWA concentrations were <0.7 (<LOQ for negative and solvent controls), 1.26, 2.15, 3.47, 5.85, and 9.87 mg a.i./L, respectively. The reviewer's results are similar to those reported by the study author; however, the reviewer's results are based on TWA concentrations, whereas the study author's were based on mean-measured concentrations.

At 96 h, mortality was 10% in the controls and the 1.26 mg a.i./L treatment levels, 20% in the 2.15 and 5.85 mg a.i./L groups, 30% in the 3.47 mg a.i./L group, and 70% in the 9.87 mg a.i./L group. The observed 96-h LC₅₀, based on the TWA concentrations, was 8.5 mg a.i./L. A NOAEC value of 1.26 mg a.i./L, based on mortality, was established; the corresponding LOAEC value was 2.15 mg a.i./L. Based on the results of this study, BAS 800 H is categorized as moderately toxic to mysid shrimp on an acute toxicity basis in accordance with the classification system of the U.S. EPA.

This toxicity study is classified as **ACCEPTABLE** to the **U.S. EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound and satisfies the guideline requirement for an acute toxicity study with saltwater mysid.

8. STUDY PARAMETERS

Age or Size of Test Organism:	Juveniles (<24 Hours Old)
Definitive Test Duration:	96 Hours
Study Method:	Flow-Through
Type of Concentrations:	Time-Weighted Average (TWA)

9. CONCLUSIONS:

Based on the results of this study, Saflufenacil is categorized as moderately toxic to mysids on an acute toxicity basis.

Results Synopsis

LC₅₀: 8.5 mg a.i./L (TWA) 95% C.I.: 7.4-11 mg a.i./L
NOAEC: 1.26 mg a.i./L (TWA)
Probit Slope: 2.51 (1.28-3.73) (based on probit method)

10. ADEQUACY OF THE STUDY

A. Classification: ACCEPTABLE to U.S. EPA; FULLY RELIABLE TO PMRA AND APVMA.

B. Rationale: N/A

C. Repairability: N/A

11. BACKGROUND

12. GUIDELINE DEVIATIONS

The TOC of the dilution water was not reported.

This deviation does not impact the acceptability of the study.

13. **SUBMISSION PURPOSE:** This study was submitted to provide data on the effects of the test material on the saltwater mysid (*Americamysis bahia*) following acute laboratory exposure for the purposes of new chemical registration.

14. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species are <i>Mysidopsis bahia</i> , <i>Penaeus setiferus</i> , <i>P. duorarum</i> , <i>P. aztecus</i> and <i>Palaemonetes sp.</i>	<i>Americamysis bahia</i>
<u>Age</u> Juvenile, mysids should be ≤ 24 hours old	<24 Hours
<u>Supplier</u>	In-house laboratory cultures
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> minimum 10 days	14 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	The adults in the culture showed no signs of disease or stress.

Guideline Criteria	Reported Information
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Feeding</u> No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each. Mysids should be fed throughout the study.	Mysids in cultures were fed live brine shrimp <i>Artemia nauplii</i> (Brine Shrimp Direct, Ogden, Utah) daily, occasionally enriched with ALGAMAC-2000 (Aquafauna Bio-Marine, Inc., Hawthorne, California). During the definitive test, juvenile mysids were fed live brine shrimp, <i>Artemia nauplii</i> , daily.
<u>Pretest Mortality</u> <3% mortality 48 hours prior to testing	No pretest mortality was reported.

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Natural seawater collected at Indian River Inlet, Delaware that was filtered and diluted to a salinity of 20‰ with well water.
Does water support test animals without observable signs of stress?	Yes
<u>Salinity</u> 30-34‰ (parts per thousand) for marine (stenohaline) shrimp and 10-17‰ for estuarine (euryhaline) shrimp, weekly range < 6‰	18 – 20‰
<u>Water Temperature</u> Approx. 22 ± 1 °C	24.7-24.9°C

Guideline Criteria	Reported Information
<u>pH</u> 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	8.0-8.1
<u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 st 48 hrs and $\geq 40\%$ during 2 nd 48 hrs, Flow-through: $\geq 60\%$	≥ 6.8 mg/L ($\geq 93\%$ saturation)
<u>Total Organic Carbon</u> Should be <5 mg/L in reconstituted seawater	Not Reported
<u>Test Aquaria</u>	
1. <u>Material</u> : Glass or stainless steel	1. Stainless steel
2. <u>Size</u> : 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp).	2. 25 L
3. <u>Fill volume</u> : 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	3. 15 L Each test chamber held a single compartment consisting of a glass container approximately 12 cm in diameter and 19 cm in height, with nylon screens attached to two holes in the sides. Test chambers were indiscriminately positioned in a temperature-controlled water bath. The water bath was enclosed in a plexiglass ventilation hood.

Guideline Criteria	Reported Information
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	Continuous-flow diluter. A syringe pump delivered the appropriate stock solution into mixing chambers containing dilution water, where the appropriate concentrations were obtained and delivered to the test vessels. The general operation of the diluter was checked visually at least twice each day during the test and at least once on the last day of the test.
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	10 vol/24 hours
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^{\circ}\text{C}$, ≤ 0.5 g/L at $> 17^{\circ}\text{C}$; flow-through: ≤ 1 g/L/day (N/A for mysids)	N/A; mysids were introduced into the vessels one or two at a time until each vessel contained 10 mysids.
<u>Photoperiod</u> 16 hours light, 8 hours dark	16L:8D with a 30 minute transition period of low light intensity. Light intensity at test initiation was 231 lux at the surface of the water of one representative test chamber.
<u>Solvents</u> Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: DMF Maximum conc.: 0.1 ml/L

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 shrimp, then no definitive test is required.	A 96-hour non-GLP range-finding study was conducted under flow-through conditions using nominal concentrations of 0 (negative and solvent controls), 0.24, 0.81, 2.7, 9.0 and 30 mg a.i./L. Following 96 hours of exposure, mortality was 0% in the controls and nominal 0.24-2.7 mg a.i./L treatment levels, and 100% in the nominal 9.0 and 30 mg a.i./L treatment levels. All test solutions appeared clear and colorless with a heavy precipitate on the bottom of the chamber at 30 mg a.i./L. Furthermore, precipitate was present in the diluter mixing cups at 9.0 and 30 mg a.i./L.
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	0 (negative and solvent controls), 1.3, 2.2, 3.5, 6.0 and 10 mg a.i./L
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers	20 per control and treatment level, equally divided between two replicates
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
<u>Water Parameter Measurements</u>	
1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1EC	1. Temperature was measured in every test vessel at 0 and 96 hours. Temperature was also measured continuously in one negative control replicate.
2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	2. DO and pH were measured in alternating replicate test chambers of each treatment and control group at the beginning and end of the test and at approximately 24 hour intervals during the test.
<u>Chemical Analysis</u> needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Concentrations of the test material in the dilution water were determined at 0, 48 and 96 hours by HPLC using a variable wavelength detector set at 240 nm.

15. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in compliance with the Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Parts 160 and 792, 17 August 1989); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau 1, October 1999), with the following exception: periodic analyses of saltwater for potential contaminants were performed using a certified laboratory and standard U.S. EPA analytical methods.
<u>Recovery of Chemical</u>	97.1-99.2% of nominal, based on the reviewer-calculated TWA concentrations (see Appendix II).
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	10% in both controls
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (mg a.i./L)		Number of Mysids	Cumulative Number Dead			
Nominal	TWA		Hour of Study			
			24	48	72	96
Control	<0.700	20	0	0	0	2
Solvent Control	<0.700	20	0	0	1	2
1.3	1.26	20	0	0	2	2
2.2	2.15	20	0	0	2	4
3.5	3.47	20	0	0	5	6
6.0	5.85	20	0	0	2	4
10	9.87	20	0	4	8	14

Other Significant Results:

Within five hours of test initiation, two mysids at the highest treatment level were observed to be surfacing. Sub-lethal effects continued at this level throughout the duration of the definitive exposure period and included lethargy. No effects were observed in any other treatment level until 48 hours, at which point one mysid in the TWA 5.85 mg a.i./L treatment group was lethargic. Also, mysids in the negative control and TWA 3.47 and 5.85 mg a.i./L treatment levels were unobserved due to possible injury by removing the test compartment from the test chamber; these mysids were assumed to be normal. By test termination at 96 hours, all surviving mysids in the controls and TWA 1.26-5.85 mg a.i./L treatment levels appeared to be normal and healthy. At the TWA 9.87 mg a.i./L treatment level, three mysids were lethargic and two mysids were observed surfacing. The study authors reported NOAEC and LC₅₀ values of 1.3 and 8.0 mg a.i./L, respectively.

B. Statistical Results

Method: The mortality data were analyzed using the computer program of C.E. Stephan. The program was designed to calculate the LC₅₀ value and the 95% confidence interval by probit analysis, the moving average method, and binomial probability with nonlinear interpolation. The study authors calculated the 96-hour LC₅₀ value using binomial probability with linear interpolation. There was less than 50% mortality in any treatment group through 72 hours of exposure. Therefore, the 24, 48 and 72-hour LC₅₀ values, as well as the no-mortality concentration and the NOAEC, were determined by visual interpretation of the mortality and observation data. All toxicity values were based on the mean-measured concentrations.

96-hr LC₅₀: 8.0 mg a.i./L

95% C.I.: >2.1 mg a.i./L

NOAEC: 1.3 mg a.i./L

Probit Slope: Not Reported

16. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	8.5 (5.85-∞) mg a.i./L
Moving Average Angle LC ₅₀ (95% C.I.)	8.5 (7.4-11) mg a.i./L
Probit LC ₅₀ (95% C.I.)	8.8 (6.4-17) mg a.i./L
Probit Slope	2.51 (1.28-3.73)
NOAEC	1.26 mg a.i./L

17. REVIEWER'S COMMENTS:

The reviewer's results were based on the time-weighted average (TWA) concentrations (refer to associated Excel worksheet in Appendix II), while those of the study authors were based on the mean-measured concentrations. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

Although the reviewer's statistical analysis of mortality indicated a NOAEC value of 5.85 mg a.i./L, it appears that the mortality at the TWA 2.15-5.85 mg a.i./L was treatment related and biologically significant. Therefore, the reviewer visually determined the NOAEC value to be 1.26 mg a.i./L.

The reviewer determined the time-weighted average concentrations using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

C_{TWA} is the time-weighted average concentration,

C_j is the concentration measured at time interval j ($j = 0, 1, 2, \dots, n$)

t_j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., $t_0 = 0$ hours (test initiation), $t_1 = 24$ hours, $t_2 = 96$ hours)

Three definitive tests were initiated under static-renewal conditions, but were terminated due to poor analytical recovery. The in-life portion of the definitive flow-through toxicity test was conducted from May 7 to 11, 2007.

17. REFERENCES:

- U.S. Environmental Protection Agency. 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1035: *Mysid Acute Toxicity Test*.
- U.S. Environmental Protection Agency. 1985. Standard Evaluation Procedure: *Acute Toxicity Test for Estuarine and Marine Organisms (Shrimp 96-Hour Acute Toxicity Test)*. Hazard Evaluation Division. Office of Pesticide Programs. EPA-540/9-85-010. Washington, DC.
- ASTM Standard E729-96. 1996. *Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians*. American Society for Testing and Materials.
- Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal Communication.
- Finney, D.J. 1971. *Statistical Methods in Biological Assay*. Second edition. Griffin

Press, London.

Thompson, W.R. 1947. *Bacteriological Reviews*. Vol II, No. 2. Pp. 115-145.

Stephan, C.E. 1977. "Methods for Calculating an LC50", *Aquatic Toxicology and Hazard Evaluations*. American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
9.87	18	12	66.6667	11.89423
5.85	18	2	11.1111	.656128
3.47	18	4.000001	22.2222	1.54419
2.15	18	2	11.1111	.0656128
1.26	18	0	0	3.814697E-04

THE BINOMIAL TEST SHOWS THAT 5.85 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 8.529939

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
1	.3121098	8.529939	7.362612 10.97545

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.2379784	1	9.079909E-02

SLOPE = 2.508756
95 PERCENT CONFIDENCE LIMITS = 1.284909 AND 3.732603

LC50 = 8.751511
95 PERCENT CONFIDENCE LIMITS = 6.354244 AND 17.11821

LC10 = 2.728074
95 PERCENT CONFIDENCE LIMITS = 1.349155 AND 3.782848

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	2	
1	1.26	20	2	
2	2.15	20	4	
3	3.47	20	6	
4	5.85	20	4	
5	9.87	20	14	*

APPENDIX II. COPY OF REVIEWER'S TWA CALCULATIONS:Time-Weighted Average (TWA)
Concentrations

Nominal (mg ai/L)	0 Hrs	% of Nom.	48 Hrs	% of Nom.	96 Hrs	% of Nom.	TWA (mg ai/L)	% of Nom.
Negative Control	<0.700	N/A	<0.700	N/A	<0.700	N/A	<0.700	N/A
Solvent Control	<0.700	N/A	<0.700	N/A	<0.700	N/A	<0.700	N/A
1.3	1.26	96.9	1.26	96.9	1.27	97.7	1.26	97.1
2.2	2.13	96.8	2.16	98.2	2.13	96.8	2.15	97.5
3.5	3.41	97.4	3.51	100.3	3.46	98.9	3.47	99.2
6.0	5.83	97.2	5.89	98.2	5.79	96.5	5.85	97.5
10	9.97	99.7	9.87	98.7	9.75	97.5	9.87	98.7